

**In the Specification**

Please enter the enclosed SEQUENCE LISTING into the specification.

Please amend the paragraph beginning at line 26 of page 23 as follows:

Further provided is a vector comprising a ribosome binding site which optionally overlaps by one nucleotide with a *SgfI* recognition site and a recognition site for a first restriction enzyme that generates blunt ends, which vector, once digested with *SgfI* and the first restriction enzyme and ligated to a DNA fragment comprising an open reading frame encoding a peptide or polypeptide flanked by

5' CGCCATGX<sub>1</sub>Y<sub>1</sub>  
3' TAGCGGTACX<sub>2</sub>Y<sub>2</sub> (SEQ ID NO:2)

and a blunt end generated by a second restriction enzyme that has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends, yields a recombinant vector which encodes the peptide or polypeptide, wherein X<sub>1</sub> is the first codon which is 3' to the start codon for the open reading frame, wherein X<sub>2</sub> is the complement of X<sub>1</sub>, wherein Y<sub>1</sub> is the remainder of the open reading frame, and wherein Y<sub>2</sub> is the complement of Y<sub>1</sub>. In one embodiment, X<sub>1</sub> = GR<sub>1</sub>R<sub>2</sub>, wherein R<sub>1</sub> or R<sub>2</sub> = A, T, C or G.

Please amend the paragraph beginning at line 9 of page 24 as follows:

Further provided is a vector comprising a first open reading frame which includes a *PmeI* recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that generates complementary single-strand DNA overhangs, which vector, once digested with *PmeI* and the first restriction enzyme, and ligated to a DNA fragment comprising a blunt end at the 5'

end of a second open reading frame and an end generated by a second restriction enzyme which generates single-strand DNA overhangs which are complementary to the single-strand DNA overhangs generated by the first restriction enzyme, yields a recombinant vector comprising a third open reading frame comprising the first and second open reading frames. In one embodiment, the third open reading frame includes  $N_1N_2N_3GTTTN_4N_5R$  (SEQ ID NO:72), wherein  $N_1N_2N_3$  and  $TN_4N_5$  are codons that do not code for a stop codon, and wherein R is one or more codons. In another embodiment, the blunt end of the DNA fragment is generated by a restriction enzyme other than *PmeI*. In yet another embodiment, the blunt end of the DNA fragment is generated by *PmeI* digestion.

Please amend the paragraph beginning at line 10 of page 32 as follows:

Figure 1. Exemplary hapaxomers (SEQ ID NOs: 16 and 20).

Please amend the paragraph beginning at line 11 of page 32 as follows:

Figures 2A-B. Examples of hapaxomers with 3' or 5' overhangs. A) The symmetry of the site recognized by *AlwNI*, a restriction enzyme that cleaves an interrupted palindrome within the recognition site. If the bases denoted "N" are ignored, the site is symmetrically equivalent to a *PvuII* site. Arrows indicate the cleavage sites on both strands. Note that a recognition and cleavage site on only one strand must be stipulated owing to the existence of a two-fold axis of symmetry. However, because cleavage by *AlwNI* results in DNA with overhangs consisting of three bases with four possibilities for each unspecified base, the sequence at the termini will be different depending on the strand. B) The *FokI* recognition and cleavage sites illustrated in both orientations (SEQ ID NOs:73-74). Because the site lacks symmetry, there are two ways to write

the bases from 5' to 3'. The cleavage sites on both strands, indicated by arrows, must be specified in order to indicate where cutting will occur. Because the cleavage sites are outside the recognition site, the single-stranded overhangs can be any set of four bases. Note that *AlwNI* generates 3' overhangs, whereas *FokI* generates 5' overhangs.

Please amend the paragraph beginning at line 31 of page 32 as follows:

Figure 5. Site frequencies of selected restriction enzymes in six species (SEQ ID NOs: 20, 55, 75-78 and 92).

Please amend the paragraph beginning at line 3 of page 33 as follows:

Figure 7. Directional cloning using *SfiI* (SEQ ID NOs: 7 and 80).

Please amend the paragraph beginning at line 7 of page 33 as follows:

Figure 11. Restriction endonucleases useful for directional cloning with *SfiI* or other restriction enzymes generating 3 base 3' overhangs (SEQ ID NOs: 7, 12, 14, 20, 79, and 81-82).

Please amend the paragraph beginning at line 11 of page 33 as follows:

Figure 13. Directional cloning using *SapI* (SEQ ID NOs: 4 and 16).

Please amend the paragraph beginning at line 16 of page 33 as follows:

Figure 16. Use of *SgfI* to generate N-terminal fusions or no fusion at the N-terminus (SEQ ID NOs: 83-85).

Please amend the paragraph beginning at line 18 of page 33 as follows:

Figure 17. Use of *PmeI* to generate C-terminal fusions including fusions with a single amino acid (SEQ ID NOs: 86-88).

Please amend the paragraph beginning at line 22 of page 33 as follows:

Figures 19A-B. N-terminal *PacI*-*SgfI* fusion site (SEQ ID NO:89-90) and C-terminal *PmeI*-*SwaI* fusion site (SEQ ID NO:91).

Please amend the paragraph beginning on line 6 of page 34 as follows:

~~Figure 22C. Luciferase expression in 3 different hosts at 25°C, t=5 hours and 21 hours.~~

Please amend the paragraph beginning on line 3 of page 85 as follows:

Moreover, the induction of luciferase activity in transformed JM109RX cells was slow compared to luciferase activity in transformed BL21(DE3) or BL21-AI cells, yet resulted in high final induction levels, e.g., high protein levels, e.g., at times  $t = 4$  hours at which RLU were 100 X greater (~~Figures 22A and C~~). Further, the use of a rhamnose-inducible system at 25°C yielded more luciferase activity than at 37°C, e.g., at least 10-70 fold more at peak (~~Figures 22A and C~~). The observed expression profile of such a system may allow for increased solubility of the expressed protein, e.g., due to increased time to fold. In addition, the rhamnose-inducible system is glucose repressible. Therefore, combinations of rhamnose and glucose may be employed to finely tune the expression profile of a gene of interest which is linked to a *rhaBAD* promoter.

Please amend Table 1 which begins at line 1 of page 44 as follows:

Table 1

<i>Alw</i> NI	↓ CAGNNNCTG ↑ GTCNNNGAC	<i>Dra</i> III	↓ CACNNNGTG ↑ GTGNNNCAC
<i>Bbs</i> I	↓ GAAGACNN (SEQ ID NO:3) ↑ CTTCTGNNNNNN	<i>Ear</i> I	↓ CTCTTCN (SEQ ID NO:4) ↑ GAGAAGNNNN
<i>Bbv</i> I	↓ GCAGCNNNNNNNN ↑ CGTCGNNNNNNNNNN (SEQ ID NO:5)	<i>Esp</i> 3 I	↓ CGTCTCN (SEQ ID NO:6) ↑ GCAGAGNNNNNN
<i>Bgl</i> I	↓ GCCNNNNNGGC ↑ CGGNNNNNCCG (SEQ ID NO:7)	<i>Fok</i> I	↓ GGATGNNNNNNNNNN ↑ CCTACNNNNNNNNNN (SEQ ID NO:8)
<i>Bsa</i> I	↓ GGTCTCN (SEQ ID NO:9) ↑ CCAGAGNNNNNN	<i>Hga</i> I	GACGCNNNNNN (SEQ ID NO:10) CTGCGNNNNNNNNNN
<i>Bsl</i> I	↓ CCNNNNNNNGG ↑ GGNNNNNNNCC (SEQ ID NO:11)	<i>Mwo</i> I	↓ GCNNNNNNNGC ↑ CGNNNNNNNCG (SEQ ID NO:12)
<i>Bsm</i> AI	↓ GTCTCN (SEQ ID NO:13) ↑ CAGAGNNNNNN	<i>Pfl</i> MI	↓ CCANNNNNTGG ↑ GGTNNNNNACC (SEQ ID NO:14)
<i>Bsm</i> FI	↓ GTCCNNNNNNNNNN ↑ CAGGGNNNNNNNNNN (SEQ ID NO:15)	<i>Sap</i> I	↓ GCTCTTCN (SEQ ID NO:16) ↑ CGAGAAGNNNN
<i>Bsp</i> MI	↓		↓

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	ACCTGNNNN (SEQ ID NO:17) TGGACGNNNNNNNNN ↑	<i>Sfa</i> NI	GCATCNNNNN (SEQ ID NO:18) CGTAGNNNNNNNNN ↑
<i>Bst</i> XI	↓ CCANNNNNTGG GGTNNNNNACC ↑ (SEQ ID NO:19)	<i>Sfi</i> I	↓ GGCCNNNNNGGCC CCGGNNNNNCCGG ↑ (SEQ ID NO:20)

Please amend Table 2 which begins at line 17 of page 45 as follows:

Table 2

<i>Alw</i> I	↓ GGATCNNNN CCTAGNNNNN (SEQ ID NO:21) ↑	A hapaxomer with an overhang of one base
<i>Bpm</i> I	↓ CTGGAGNNNNNNNNNNNNNNNN GACCTCNNNNNNNNNNNNNNNN (SEQ ID NO:22) ↑	A hapaxomer with an overhang of two bases
<i>Bsp</i> 1286 I	G C ↓ G A G C A C T T C G C T C G T G ↑A A	An honorary hapaxomer

Please amend the table which begins at line 1 of page 73 as follows:

Enzymes	Recognition Sequence	Stop Codons w/PmeI	Codon(AA) fusion	Isoschizomers
<u>AhaIII</u>	TTT^AAA	TAA	None	DraI PauAII SruI

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<u>Enzymes</u>	<u>Recognition Sequence</u>	<u>Stop Codons w/PmeI</u>	<u>Codon(AA) fusion</u>	<u>Isoschizomers</u>
<u>AluI</u>	AG <sup>^</sup> CT	No	TCT(S) (SEQ ID NO:24)	MltI
<u>BalI</u>	TGG <sup>^</sup> CCA	No	TCC(S)ANN(IMTNKSR) (SEQ ID NO:25)	MlsI Mlu31I MluNI MscI Msp20I
<u>BfrBI</u>	ATG <sup>^</sup> CAT	No	TCA(S)TNN(FLSYC) (SEQ ID NO:26)	-
<u>BsaAI</u>	YAC <sup>^</sup> GTR	No	TGT(C)RNN(IMVTANKDESRG) (SEQ ID NO:27)	BstBAI MspYI PsuAI
<u>BsaBI</u>	GATNN <sup>^</sup> NNATC (SEQ ID NO:23)	TAA, TAG, TGA	TNN(FLSYCW)ATC(I) (SEQ ID NO:28)	Bse8I BseJI Bsh1365I BsiBI BsrBRI MamI
<u>BsrBI</u>	CCGCTC (-3/-3)	No	TCT(S)CNN(LPHQR) (SEQ ID NO:29)	AccBSI BstD102I Bst31NI MbiI
<u>BtrI</u>	CACGTC (-3/-3)	No	TGT(C)CNN(LPHQR) (SEQ ID NO:30)	BmgBI
<u>Cac8I</u>	GCN <sup>^</sup> NGC	TAG	TNG(LSW)CNN(LPHQR) (SEQ ID NO:31)	BstC8I
<u>CdiI</u>	CATCG (-1/-1)	TGA	TGN(C ) (SEQ ID NO:32)	-
<u>CviJI</u>	RG <sup>^</sup> CY	No	TCY(S) (SEQ ID NO:33)	CviTI
<u>CviRI</u>	TG <sup>^</sup> CA	No	TCA(S) (SEQ ID NO:34)	HpyCH4V HpyF44III
<u>Eco47III</u>	AGC <sup>^</sup> GCT	No	TGC(C)TNN(FLSYC) (SEQ ID NO:25)	AfeI AitI Aor51HI FunI
<u>Eco78I</u>	GGC <sup>^</sup> GCC	No	TGC(C)CNN(LPHQR) (SEQ ID NO:36)	EgeI EheI SfoI
<u>EcoICRI</u>	GAG <sup>^</sup> CTC	No	TCT(S)CNN(LPHQR) (SEQ ID NO:37)	BpuAmI Ecl136II Eco53kI MxaI
<u>EcoRV</u>	GAT <sup>^</sup> ATC	No	TAT(Y)CNN(LPHQR) (SEQ ID NO:38)	CeqI Eco32I HjaI HpyCI NsiCI
<u>EsaBC3I</u>	TC <sup>^</sup> GA	TGA	None	-
<u>FnuDII</u>	CG <sup>^</sup> CG	No	TCG(S) (SEQ ID NO:39)	AccII BceBI BepI Bpu95I Bsh1236I Bsp50I Bsp123I BstFNI BstUI Bsu1532I BtkI Csp68KVI CspKVI FallI FauBII MvnI ThaI
<u>FspAI</u>	RTGC <sup>^</sup> GCA Y	No	TGC(C)AYN(IMT) (SEQ ID NO:41)	-
<u>HaeI</u>	WGG <sup>^</sup> CCW	No	TCC(S)WNN(IMTNKSRFLYC) (SEQ ID NO:42)	-

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Enzymes	Recognition Sequence	Stop Codons w/PmeI	Codon(AA) fusion	Isoschizomers
<u>HaeIII</u>	FspAIGG^CC	No	TCC(S) (SEQ ID NO:43)	BanAI BecAII BimI9II Bme361I BseQI BshI BshFI Bsp211I BspBRI BspKI BspRI BsuRI BteI CtiI DsaII EsaBC4I FnuDI MchAII MfoAI NgoPII NspLKI PalI Pde133I PfiKI PhoI PlaI SbvI SfaI SuiI
<u>HindII</u>	GTY^RAC	TAA, TGA	None	HinJCI HincII
<u>HpaI</u>	GTT^AAC	TAA	None	BstEZ359I BstHPI KspAI SsrI
<u>Hpy8I</u>	GTN^NAC	TAA, TGA	TYA(FLS)CNN((LPHQR) (SEQ ID NO:44)	HpyBII
<u>LpnI</u>	RGC^GCY	No	TGC(C)YNN(FLSYCLPHQR) (SEQ ID NO:45)	Bme142I
<u>MlyI</u>	GAGTC (5/5)	TAA, TAG, TGA	Any	SchI
<u>MslI</u>	CAYNN^NNRTG (SEQ ID NO:40)	TAA, TAG, TGA	TNN(FLSYCW)RTG(MV) (SEQ ID NO:46)	SmiMI
<u>MstI</u>	TGC^GCA	No	TGC(C)ANN(IMTNKSR) (SEQ ID NO:47)	Acc16I AoiI AviII FdiII FspI NsbI PamiI Pun14627I
<u>NaeI</u>	GCC^GGC	No	TGG(C)CNN(LPHQR) (SEQ ID NO:48)	CcoI PdiI SauBMKI SauHPI SauLPI SauNI SauSI Slu1777I SspCI
<u>NlaIV</u>	GGN^NCC	No	TNC(FSYC)CNN(LPHQR) (SEQ ID NO:49)	AspNI BscBI BspLI PspN4I
<u>NruI</u>	TCG^CGA	No	TCG(S)ANN(IMTNKSR) (SEQ ID NO:50)	Bsp68I MluB2I Sbo13I SpoI
<u>NspBII</u>	CMG^CKG	No	TCK(S)GNN(VA DEG) (SEQ ID NO:51)	MspAII
<u>OliI</u>	CACNN^NNGTG	TAA, TAG, TGA	TNN(FLSYCW)GTG(V) (SEQ ID NO:52)	AleI
<u>PmaCI</u>	CAC^GTG	No	TGT(S)GNN(VA DEG) (SEQ ID NO:53)	AcvI BbrPI BcoAI Eco72I PmlI



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Enzymes	Recognition Sequence	Stop Codons w/PmeI	Codon(AA) fusion	Isoschizomers
<u>PmeI</u>	GTTT^AAAC	TAA	None	MssI
<u>PshAI</u>	GACNN^NNGTC (SEQ ID NO:54)	TAA, TAG, TGA	TNN(FLSYCW)GTC(V) (SEQ ID NO:56)	BoxI BstPAI
<u>PsiI</u>	TTA^TAA	No	TTA(L)ANN(IMTNKSR) (SEQ ID NO:68)	-
<u>PvuII</u>	CAG^CTG	No	TCT(S)GNN(VA DEG) (SEQ ID NO:57)	BavI BavAI BavBI Bsp153AI BspM39I BspO4I Cfr6I DmaI EclI NmeRI Pae17kI Pun14627II Pvu84II Uba153AI UbaM39I
<u>RsaI</u>	GT^AC	No	TAC(Y) (SEQ ID NO:58)	AfaI HpyBI PlaAII
<u>ScaI</u>	AGT^ACT	No	TAC(Y)TNN(FLSYCW) (SEQ ID NO:59)	AccI13I AssI DpaI Eco255I RflFII
<u>SciI</u>	CTC^GAG	TGA	None	-
<u>SmaI</u>	CCC^GGG	No	TGG(C)GNN(VA DEG) (SEQ ID NO:60)	CfrJ4I PaeBI PspALI
<u>SnaBI</u>	TAC^GTA	No	TGT(S)ANN(IMTNKSR) (SEQ ID NO:61)	BstSNI Eco105I
<u>SrfI</u>	GCCC^GGGC	No	TGG(C)GCN(A) (SEQ ID NO:62)	-
<u>SspI</u>	AAT^ATT	No	TAT(Y)ANN(IMTNKSR) (SEQ ID NO:63)	-
<u>SspD5I</u>	GGTGA (8/8)	TAA, TAG, TGA	Any	-
<u>StuI</u>	AGG^CCT	No	TCC(S)TNN(FLSYCW) (SEQ ID NO:64)	AatI AspMI Eco147I GdiI PceI Pme55I SarI Sru30DI SseBI SteI
<u>SwaI</u>	ATTT^AAAT	TAA	None	BstRZ246I BstSWI MspSWI SmiI
<u>XcaI</u>	GTA^TAC	No	TTA(L)CNN(LPHQR) (SEQ ID NO:65)	BspM90I BssNAI Bst1107I BstBSI BstZ17I
<u>XmnI</u>	GAANN^NNTTC (SEQ ID NO:55)	TAA, TAG, TGA	TNN(FLSYCW)TTC(F) (SEQ ID NO:66)	Asp700I BbvAI MroXI PdmI

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<u>ZraI</u>	GAC^GTC	No	TGT(S)CNN(LPHQR) (SEQ ID NO:67)	-